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Molecular Characterization and Epidemiology of Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolates Causing Health Care-Associated Infection in Thailand, Where the CTX-M Family Is Endemic▽†

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Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* have rapidly spread worldwide and pose a serious threat for health care-associated (HA) infection. We conducted molecular detection and characterization of ESBL-related *bla* genes, including bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{VEB}, bla_{OXA}, bla_{PER}, and bla_{GES}, among 362 isolates of ESBL-producing *E. coli* ($n = 235$) and ESBL-producing *K. pneumoniae* ($n = 127$) collected from patients who met the definition of HA infection at two major university hospitals in Thailand from December 2004 to May 2005. The prevalence of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae*, patient demographics and the susceptibilities of these bacteria to various antimicrobial agents were described. A total of 87.3% of isolates carried several *bla* genes. The prevalence of bla_{CTX-M} was strikingly high: 99.6% for ESBL-producing *E. coli* (CTX-M-14, -15, -27, -40, and -55) and 99.2% for ESBL-producing *K. pneumoniae* (CTX-M-3, -14, -15, -27, and -55). IS*Ecp1* was found in the upstream region of bla_{CTX-M} in most isolates. Up to 77.0% and 71.7% of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae*, respectively, carried bla_{TEM}; all of them encoded TEM-1. ESBL-producing *K. pneumoniae* carried bla_{SHV} at 87.4% (SHV-1, -2a, -11, -12, -27, -71, and -75) but only at 3.8% for ESBL-producing *E. coli* (SHV-11 and -12). *bla* genes encoding VEB-1 and OXA-10 were found in both ESBL-producing *E. coli* (8.5% and 8.1%, respectively) and ESBL-producing *K. pneumoniae* (10.2% and 11.8%, respectively). None of the isolates were positive for bla_{PER} and bla_{GES}. Pulsed-field gel electrophoresis analysis demonstrated that there was no major clonal relationship among these ESBL producers. This is the first study to report CTX-M-3, CTX-M-27, CTX-M-40, SHV-27, SHV-71, and SHV-75 in Thailand and to show that CTX-M ESBL is highly endemic in the country.

Extended-spectrum β-lactamases (ESBLs) have spread threateningly in many regions of the world and presently comprise over 300 variants (http://www.lahey.org/Studies). A typical characteristic of ESBLs is their ability to hydrolyze oxyiminocephalosporins and aztreonam while being inhibited by β-lactamase inhibitors (8, 30). ESBL-producing organisms have achieved notoriety for causing nosocomial infections (7, 30). Based on multivariable analysis, it was shown that exposure to oxyiminocephalosporins and transfer from another hospital were among identified risk factors associated with infection due to ESBL producers in Thailand (2). Therefore, health care interactions appeared to play a role in the acquisition of ESBL producers.

The first ESBL, SHV-2, was reported from a strain of *Klebsiella ozaenae* in 1983 (21). Thereafter, the number of ESBL variants occurring through amino acid mutations has progressively increased while demonstrating geographic variations (38). SHV-type ESBLs are mostly derivatives of a non-ESBL SHV-1 and quickly invaded several continents (7, 29). The majority of plasmid-mediated β-lactamases, namely, TEM-1 or, less frequently, TEM-2, are broad-spectrum β-lactamases, which, in contrast to ESBLs, do not hydrolyze oxyiminocephalosporins or aztreonam. Several TEM-1 derivatives, however, confer ESBL properties and are prevalent in North America (30). The CTX-M family, first described in 1992 (3), is known to be the most dominant non-TEM, non-SHV ESBL among *Enterobacteriaceae* and is recognized as a rapidly growing family of ESBLs that selectively prefer to hydrolyze cefotaxime rather than ceftazidime (6). However, variants of CTX-M with increased hydrolyzing activity against ceftazidime have emerged (20, 31, 32). The insertion sequence (IS) element IS*Ecp1* is commonly present in the upstream region of the CTX-M gene and is likely responsible for the transposition process of the gene (34). Other ESBL enzymes are less often encountered. VEB-1 ESBL was identified among *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates and was previously reported from Thailand (14, 15). Among members of the OXA family, a class D β-lactamase originating in *P. aeruginosa*, derivatives of OXA-2 and -10 show activity considered to be ESBL like. The PER and GES families, both first identified in *P. aeruginosa*, are also among β-lactamase families exhibiting ESBL-like activities. Nevertheless, epidemiologic data on these less common ESBLs are very limited. Several surveillance studies

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TABLE 1. Primers used for PCR amplification of *bla* genes and IS element

Target	Primer name	Primer sequence (5′–3′)	Product size (bp)	Reference(s)
bla_{TEM}	TEM-F	TCCGCTCATGAGACAATAACC	931	35
	TEM-R	TTGGTCTGACAGTTACCAATGC		
bla_{SHV}	SHV-F	TGGTTATGCGTTATATTCGCC	868	27
	SHV-R	GGTTAGCGTTGCCAGTGCT		
bla_{CTX-M}	CTX-F	TCTTCCAGAATAAGGAATCCC	909	35
	CTX-R	CCGTTTCCGCTATTACAAAC		
bla_{VEB}	VEB-F1	GATAGGAGTACAGACATATG	914	28
	VEB-R1	TTTATTCAAATAGTAATTCCACG		
bla_{OXA-2} group	OXA-2-F	AAGAAACGCTACTCGCCTGC	478	40
	OXA-2-R	CCACTCAACCCATCCTACCC		
bla_{OXA-10} group	OXA-10-F	GTCTTTCGAGTACGGCATTA	720	5
	OXA-10-R	ATTTTCTTAGCGGCAACTTAC		
bla_{PER}	PER-F	ATGAATGTCATCACAAAATG	927	10
	PER-R	TCAATCCGGACTCACT		
bla_{GES}	GES-F	ATGCGCTTCATTCACGCAC	864	37
	GES-R	CTATTTGTCCGTGCTCAGG		
IS*Ecp1* element	IS-F	GTGCCCAAGGGGAGTGTATG	615	20, 34
	IS-R	ACYTTACTGGTRCTGCACAT		

revealed a relatively high prevalence of ESBL-producing organisms in the Asia-Pacific area (4, 17). In particular, CTX-M ESBL is believed to be the dominant type in East Asia, since it has appeared or caused outbreaks in many countries (6, 22). In Thailand, however, studies pertaining to molecular characterization of ESBL-conferring *bla* genes and the epidemiology of ESBL producers, especially among health care-associated (HA) infections, are rare. We report here the first extensive phenotypic and genetic studies of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* causing HA infections in Thailand, as well as patient demographics, the epidemiology of ESBL genes, and molecular typing of isolates by pulsed-field gel electrophoresis.

MATERIALS AND METHODS

Study setting, patient demographics, and clinical specimens. This study included all clinical specimens submitted for bacterial culture at the microbiology laboratories of two major university hospitals in Thailand, the 2,400-bed Siriraj hospital (Bangkok) and the 500-bed Thammasart hospital (Pratumthani province, north of Bangkok) from December 2004 to May 2005. ESBL producers are not limited to causing nosocomial infections; community-onset infections have also been reported in Thailand (1, 20). Most community-onset cases, however, have been related to health care. In this study, we focused on patients who had HA infections. An HA infection was previously defined (2) as an infection that met one of the following criteria: (i) it occurred more than 48 h after admission to a hospital, (ii) it occurred 48 h or less after admission in a patient who had been hospitalized during the 30 days prior to admission to our hospitals, or (iii) it occurred in a patient who was transferred from another hospital or nursing home. All patients who had infections due to ESBL-producing *E. coli* or ESBL-producing *K. pneumoniae* and met the definition of HA infection were reviewed for their demographics, including age, sex, the hospital unit where they had received medical service, and the types of clinical specimens. The χ^2 test was used where appropriate to compare discrete variables, which were considered statistically significant at a P value of <0.05. This study was approved by the ethics committees of both institutions.

Bacterial isolates, antimicrobial susceptibility testing, and detection of ESBL. Bacteria recovered from clinical specimens were identified by standard biochemical methods. The production of ESBL was tested for all *E. coli* and *K. pneumoniae* isolates by using the combination disk method based on the inhibitory effect of clavulanic acid according to the Clinical and Laboratory Standards Institute (CLSI) criteria (13). Consecutive nonduplicate clinical isolates of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* were collected for further investigation. The MIC_{50} and MIC_{90} values of six antimicrobial agents (ceftazidime, cefotaxime, ceftriaxone, imipenem, ertapenem, and meropenem) for ESBL-producing isolates were determined by the Etest method (AB Biodisk, Solna, Sweden). All isolates were also tested against non-β-lactam agents, including tetracycline, amikacin, gentamicin, cotrimoxazole, and ciprofloxacin, by the standard disk diffusion method, and the results were interpreted based on the CLSI guidelines (13). Isolates shown to be resistant to at least three different classes of antimicrobial agents were determined to be multidrug resistant (MDR). All antimicrobial disks used for susceptibility testing were obtained from BD BBL Sensi-Disc (Becton Dickinson, Sparks, MD). *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used for quality control processes as recommended by CLSI (13).

DNA manipulation, PCR, and nucleotide sequencing. Because most ESBL genes were shown to be plasmid mediated, plasmid DNA was extracted and purified using the Nucleospin Plasmid kit (Macherey-Nagel, Düren, Germany). PCR amplification of *bla* genes, including bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{VEB}, bla_{OXA}, bla_{PER}, and bla_{GES}, was performed with *Taq* DNA polymerase (Invitrogen, Carlsbad, CA) using primers listed in Table 1. PCR detection of bla_{OXA} was focused on the bla_{OXA-2} and bla_{OXA-10} groups, whose derivatives have shown ESBL characteristics. The PCRs were conducted in a GeneAmp PCR system 9600 (Perkin-Elmer, Norwalk, CT) under the conditions specified in the reference sources (Table 1). Detection of IS elements in the upstream region of bla_{CTX-M} was performed for all isolates carrying bla_{CTX-M} by PCR using primers and conditions described previously (20, 34). All PCR amplicons were verified by gel electrophoresis on a 2.0% (wt/vol) SeaKem LE agarose gel (FMC Bio Products, Rockland, ME). The gels were stained with ethidium bromide (0.5 μg/ml). Purifications of PCR amplicons were carried out by using a Nucleospin Extract II kit (Macherey-Nagel). PCR products of *bla* genes and IS elements were subjected to bidirectional nucleotide sequencing using PCR primers to determine their molecular types. DNA sequences were analyzed with the 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations.

Conjugation assay. To demonstrate that *bla* genes detected by PCR were located on plasmids, a resistance transfer experiment using a conjugation assay (broth mating) was performed for selected isolates that carried at least four *bla* genes as described previously (16). *E. coli* JM109 was used as the recipient. Transconjugants were selected on MacConkey agar (Oxoid, Basingstoke, United Kingdom) supplemented with cefotaxime (2 mg/liter). Antimicrobial susceptibility, a confirmatory test for ESBL phenotype, PCR detection, and DNA sequencing of *bla* genes were performed for transconjugants by the procedures mentioned above.

Pulsed-field gel electrophoresis (PFGE). For molecular typing, chromosomal DNA of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* were obtained for comparison by PFGE analysis using a CHEF Mapper XA apparatus (Bio-Rad Laboratories, Hercules, CA). Agarose plugs containing bacterial DNA were prepared and processed for PFGE as described elsewhere (19). Restriction analysis of chromosomal DNA with XbaI (New England BioLabs, Beverly, MA) was performed, and separation of DNA was carried out by using 1% pulsed-field gel agarose (Bio-Rad Laboratories, La Jolla, CA). DNA banding patterns were

TABLE 2. Demographics of and specimen types from patients who had HA infections due to ESBL-producing *E. coli* or *K. pneumoniae*

Parameter	Value	% of patients	
		E. coli (n = 235)	*K. pneumoniae* (n = 127)
Sex	Male/female	38/62	47/53
Age group (yr)	≤20	11	16
	21–40	12	9
	41–60	22	13
	>60	55	62
Hospital unit	Medicine	32	33
	Surgery	34	27
	Pediatrics	8	11
	ICU[a]	8	20
	Outpatient	16	9
	Other	2	0
Specimen type	Blood	8	11
	Sputum	12	16
	Urine	63	56
	Pus/exudate	17	17

[a] ICU, intensive-care units.

compared using Fingerprinting II Software, version 3.0 (Bio-Rad Laboratories, Hercules, CA). The unweighted pair group method using arithmetic averages was used to generate a dendrogram with the position tolerances set at 1.5%. The Dice coefficients were used for band similarity measurements to determine DNA relatedness. Isolates were considered to be clonally related if the Dice coefficient correlation was over 80%, which corresponds to the "possibly related" category according to the criteria suggested by Tenover et al. (36).

RESULTS

Prevalence of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* and patient demographics. During the study period, a total of 2,777 patients were identified as having infections due to *E. coli* (n = 1,776) or *K. pneumoniae* (n = 1,001) at both major tertiary-care centers. Among these patients, 235 and 127 had infections due to ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae*, respectively, and met the criteria for HA infection. Thus, the prevalences of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* causing HA infections were 13.2% and 12.7%, respectively. Patient demographics are shown in Table 2. Female patients predominated, particularly for ESBL-producing *E. coli* infection. A majority of patients were over 60 years old. Most patients had been hospitalized in medical or surgical units. ESBL-producing *K. pneumoniae* was more dominant among patients admitted to intensive-care units, while ESBL-producing *E. coli* was more common in outpatient departments. For both ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae*, a majority of isolates were recovered from urine specimens.

Susceptibility of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* to antimicrobial agents. The MIC_{50}s/MIC_{90}s (μg/ml) of ceftazidime, ceftriaxone, cefotaxime, imipenem, ertapenem, and meropenem for ESBL-producing *E. coli* were 32/256, >256/>256, 256/>256, 0.25/0.38, 0.094/0.38, and 0.047/0.094, respectively, and those for ESBL-producing *K. pneumoniae* were 128/>256, 256/>256, 128/>256, 0.25/0.38, 0.094/0.38, and 0.047/0.125, respectively. All isolates demonstrated high MICs of oxyiminocephalosporin while they remained in the susceptible range for carbapenems. The rates of resistance to non-β-lactam agents, including tetracycline, amikacin, gentamicin, cotrimoxazole, and ciprofloxacin, for ESBL-producing *E. coli* were 87.5%, 12.5%, 66.3%, 72.7%, and 78.1%, respectively, and for ESBL-producing *K. pneumoniae* they were 85.7%, 28.6%, 76.4%, 72.1%, and 76.6%, respectively. Up to 72.3% and 81.9% of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae*, respectively, expressed the MDR phenotype.

TABLE 3. Molecular characterization of *bla* genes among ESBL-producing *E. coli* isolates (n = 235)

Genotype of *bla* gene[a]	No. of isolates
CTX-M-14	15
CTX-M-14, TEM-1	69
CTX-M-14, TEM-1, SHV-11	2
CTX-M-14, TEM-1, SHV-12	3
CTX-M-14, TEM-1, SHV-12, OXA-10, VEB-1	1
CTX-M-14, VEB-1	1
CTX-M-14, OXA-10, VEB-1	4
CTX-M-14, TEM-1, OXA-10, VEB-1	7
CTX-M-15	25
CTX-M-15, TEM-1	55
CTX-M-15, SHV-11	1
CTX-M-15, SHV-12	1
CTX-M-15, TEM-1, SHV-12	1
CTX-M-15, OXA-10, VEB-1	1
CTX-M-15, TEM-1, OXA-10, VEB-1	3
CTX-M-27	1
CTX-M-27, TEM-1	2
CTX-M-40, OXA-10, VEB-1	1
CTX-M-55	3
CTX-M-55, TEM-1	37
CTX-M-55, TEM-1, OXA-10, VEB-1	1
OXA-10, VEB-1	1

[a] TEM-1, SHV-1, SHV-11, and OXA-10 are not ESBL.

Molecular characterization of ESBL genes. All ESBL-producing isolates were subjected to PCR experiments to detect ESBL genes, including bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{VEB}, bla_{OXA} (OXA-2 and OXA-10 groups), bla_{PER}, and bla_{GES}. Only bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{VEB}, and bla_{OXA-10} groups were detected in 77.0%, 3.8%, 99.6%, 8.5%, and 8.1% of ESBL-producing *E. coli* and 71.7%, 87.4%, 99.2%, 10.2%, and 11.8% of ESBL-producing *K. pneumoniae*, respectively (Tables 3 and 4). None of the bla_{OXA-2} group, bla_{PER}, and bla_{GES} were present in all isolates. Three hundred and sixteen out of 362 isolates (87.3%) carried several *bla* genes (up to five genes). bla_{CTX-M} was most prevalent in both ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae*. Among ESBL-producing *E. coli* isolates, bla_{CTX-M} encoding CTX-M-14, -15, -27, -40, and -55 was found in 43.6%, 37.2%, 1.3%, 0.4%, and 17.5% of isolates, respectively. Among ESBL-producing *K. pneumoniae* isolates, bla_{CTX-M} encoding CTX-M-3, -14, -15, -27, and -55 was found in 3.2%, 52.4%, 38.9%, 0.8%, and 4.7% of isolates, respectively. IS*Ecp1* was present in the upstream region of bla_{CTX-M} in 357 isolates (99.2%). Upon sequencing of the bla_{CTX-M} upstream DNA, IS*10*, IS*26*, and IS*903* were also found interrupting IS*Ecp1* in two, nine, and five isolates, respectively. The structural analysis of IS*Ecp1*-carrying bla_{CTX-M} upstream DNA is shown in Table 5. bla_{SHV} genes from ESBL-producing *E. coli* encoded SHV-11 and -12 at 1.2% and 2.6%, respectively, while those from ESBL-producing *K. pneumoniae*

TABLE 4. Molecular characterization of *bla* genes among ESBL-producing *K. pneumoniae* (n = 127)

Genotype of *bla* gene[a]	No. of isolates
CTX-M-3, TEM-1	1
CTX-M-3, TEM-1, OXA-10	1
CTX-M-3, TEM-1, OXA-10, VEB-1	2
CTX-M-14	2
CTX-M-14, TEM-1	6
CTX-M-14, SHV-1	1
CTX-M-14, TEM-1, SHV-1	4
CTX-M-14, SHV-2a	2
CTX-M-14, TEM-1, SHV-2a	2
CTX-M-14, SHV-11	3
CTX-M-14, TEM-1, SHV-11	4
CTX-M-14, TEM-1, SHV-11, OXA-10	1
CTX-M-14, TEM-1, SHV-11, OXA-10, VEB-1	2
CTX-M-14, SHV-12	9
CTX-M-14, TEM-1, SHV-12	23
CTX-M-14, TEM-1, SHV-12, OXA-10, VEB-1	2
CTX-M-14, TEM-1, SHV-27	1
CTX-M-14, SHV-71	2
CTX-M-14, TEM-1, OXA-10, VEB-1	1
CTX-M-15, TEM-1	2
CTX-M-15, SHV-1	4
CTX-M-15, TEM-1, SHV-1	14
CTX-M-15, SHV-1, OXA-10, VEB-1	1
CTX-M-15, SHV-11	4
CTX-M-15, TEM-1, SHV-11	12
CTX-M-15, TEM-1, SHV-11, OXA-10, VEB-1	2
CTX-M-15, SHV-12	4
CTX-M-15, TEM-1, SHV-12	2
CTX-M-15, TEM-1, SHV-12, OXA-10, VEB-1	1
CTX-M-15, TEM-1, SHV-27	1
CTX-M-15, SHV-75	1
CTX-M-15, TEM-1, SHV-75, OXA-10, VEB-1	1
CTX-M-27, TEM-1, SHV-12	1
CTX-M-55, TEM-1	1
CTX-M-55, SHV-1	1
CTX-M-55, TEM-1, SHV-1	1
CTX-M-55, SHV-11	1
CTX-M-55, TEM-1, SHV-11	2
CTX-M-55, SHV-12, OXA-10, VEB-1	1
TEM-1, SHV-12	1

[a] TEM-1, SHV-1, SHV-11, and OXA-10 are not ESBL.

encoded SHV-1, -2a, -11, -12, -27, -71, and -75 at 20.5%, 3.1%, 24.4%, 34.6%, 1.6%, 1.6%, and 1.6%, respectively. All PCR products of bla_{TEM}, bla_{VEB}, and bla_{OXA-10} groups encoded TEM-1, VEB-1, and OXA-10, respectively. TEM-1, SHV-1, SHV-11, and OXA-10 are not classified as ESBLs. Based on the conjugation assay of selected isolates, all *bla* genes detected were able to be transferred to transconjugants, suggesting that they were plasmid mediated.

TABLE 5. Analysis of the bla_{CTX-M} upstream DNA region

Type of bla_{CTX-M}	IS element	Size of intergenic spacer region between IS*Ecp1* and bla_{CTX-M} (bp)	No. of isolates
CTX-M-3	IS*Ecp1*	127	4
CTX-M-14	IS*Ecp1*	42	164
CTX-M-14	IS*10*-interrupting IS*Ecp1*	42	2
CTX-M-15	IS*Ecp1*	48	121
CTX-M-15	IS*26*-interrupting IS*Ecp1*	48	9
CTX-M-15	IS*903*-interrupting IS*Ecp1*	48	5
CTX-M-27	IS*Ecp1*	42	4
CTX-M-40	IS*Ecp1*	66	1
CTX-M-55	IS*Ecp1*	48	47

TABLE 6. Antimicrobial susceptibilities of isolates carrying bla_{CTX-M} in the CTX-M-1 and CTX-M-9 subgroups

Susceptibility to antimicrobial agents	Value			
	CTX-M-1 subgroup		CTX-M-9 subgroup	
	No other *bla* (n = 27)	With other *bla* (n = 162)	No other *bla* (n = 18)	With other *bla* (n = 152)
MIC_{90} (μg/ml)				
Ceftazidime	256	>256	16	>256[a]
Ceftriaxone	>256	>256	>256	>256
Cefotaxime	>256	>256	128	256
Imipenem	0.5	0.38	0.5	0.38
Ertapenem	0.38	0.5	0.19	0.25
Meropenem	0.19	0.125	0.094	0.064
% Susceptibility				
Tetracycline	4.8	4.9	16.7	5.9[a]
Amikacin	81.0	81.0	83.3	78.9
Gentamicin	24.1	19.0	33.3	30.3
Cotrimoxazole	14.3	14.1	31.6	8.3[a]
Ciprofloxacin	20.4	11.1[a]	25.7	8.3[a]

[a] Susceptibility decreased significantly ($P < 0.05$) compared to the value in the absence of other *bla* genes.

Antimicrobial susceptibilities of CTX-M-producing isolates. Based on amino acid sequence similarities, CTX-M-3, -15, and -55 are classified in the CTX-M-1 subgroup, while CTX-M-14 and -27 are members of the CTX-M-9 subgroup. Therefore, CTX-M-producing isolates identified in this series mainly produced ESBLs in either the CTX-M-1 or -9 subgroup. Analyses of MIC_{90}s and susceptibility patterns of these two subgroups are shown in Table 6. Without other *bla* genes, the CTX-M-1 subgroup exhibited a level of resistance to ceftazidime fourfold-higher than what appeared for the CTX-M-9 subgroup. The MIC_{90} of ceftazidime among the CTX-M-9 subgroup was elevated but remained in the susceptible range. Nevertheless, when other *bla* genes were present, the CTX-M-9 subgroup showed a significant increase in the MIC_{90} of ceftazidime to a level similar to that of the CTX-M-1 subgroup. Both subgroups demonstrated no significant difference in MICs of other β-lactam agents. Interestingly, isolates carrying the CTX-M-1 subgroup alone significantly exhibited reduced susceptibilities to tetracycline and cotrimoxazole compared to the CTX-M-9 subgroup ($P < 0.05$). In addition, when other *bla* genes were detected, the susceptibilities to ciprofloxacin and, for the CTX-M-9 subgroup only, tetracycline and cotrimoxazole were significantly decreased.

PFGE typing. To study the clonal relationships of ESBL-producing isolates, molecular typing by PFGE was analyzed for both ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae*. Chromosomal DNAs of 229 ESBL-producing *E. coli* and 127 ESBL-producing *K. pneumoniae* isolates were available for PFGE typing. Banding patterns were not obtained from six ESBL-producing *E. coli* isolates because their DNAs were consistently autodigested. The results showed that there were few isolates demonstrating DNA relatedness, and only isolates producing the same type of CTX-M exhibited such

TABLE 7. PFGE profiles of ESBL-producing *E. coli* and *K. pneumoniae* based on CTX-M type

Organism	CTX-M type	PFGE profile[a]
ESBL-producing *E. coli* ($n = 229$)	CTX-M-14	14E-1 ($n = 2$), 14E-2 ($n = 2$), 14E-3 ($n = 2$), 14E-NR ($n = 92$)
	CTX-M-15	15E-1A ($n = 9$), 15E-1B ($n = 4$), 15E-1C ($n = 3$), 15E-1D ($n = 3$), 15E-1E ($n = 2$), 15E-2 ($n = 4$), 15E-3 ($n = 3$), 15E-4 ($n = 2$), 15E-NR ($n = 56$)
	CTX-M-27	27E-NR ($n = 3$)
	CTX-M-40	40E-NR ($n = 1$)
	CTX-M-55	55E-1A ($n = 3$), 55E-1B ($n = 2$), 55E-2 ($n = 2$), 55E-3 ($n = 2$), 55E-NR ($n = 31$)
	Non-CTX-M	XE-NR ($n = 1$)
ESBL-producing *K. pneumoniae* ($n = 127$)	CTX-M-3	3K-1 ($n = 2$), 3K-NR ($n = 2$)
	CTX-M-14	14K-1A ($n = 6$), 14K-1B ($n = 5$), 14K-2A ($n = 3$), 14K-2B ($n = 2$), 14K-3 ($n = 2$), 14K-4 ($n = 2$), 14K-5 ($n = 2$), 14K-6 ($n = 2$), 14K-7 ($n = 2$), 14K-8 ($n = 2$), 14K-NR ($n = 38$)
	CTX-M-15	15K-1A ($n = 4$), 15K-1B ($n = 4$), 15K-2A ($n = 4$), 15K-2B ($n = 2$), 15K-2C ($n = 2$), 15K-3A ($n = 3$), 15K-3B ($n = 3$), 15K-4 ($n = 2$), 15K-5 ($n = 2$), 15K-NR ($n = 23$)
	CTX-M-27	27K-NR ($n = 1$)
	CTX-M-55	55K-NR ($n = 6$)
	Non-CTX-M	XK-NR ($n = 1$)

[a] Isolates in each PFGE profile exhibited >80% similarity. PFGE profiles of the following clusters showed >60 to 80% similarity to each other: 15E-1A, 15E-1B, 15E-1X, 15E-1Δ, and 15E-1E; 55E-1A and 55E-1B; 14K-1Aand 14K-1B; 14K-2A and 14K-2B; 15K-1A and 15K-1B; 15K-2A, 15K-2B, and 15K-2X; and 15K-3A and 15K-3B. NR, when in a PFGE profile, indicates a group of unrelated isolates with ≤60% similarity.

genetic relationships (see the supplemental material). Therefore, based on CTX-M types, all clusters according to PFGE profiles of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* are shown in Table 7. Clonality was determined if isolates showed over 80% similarity of PFGE patterns. In addition, there were some clusters among both ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* that exhibited over 60%, but less than 80%, similar banding patterns, suggesting that these clusters may be distantly related. Upon a retrospective chart review, all of these related isolates occurred sporadically and randomly within different hospital units and were recovered from a variety of specimen sources. A majority of isolates showed unique, unrelated PFGE profiles (Table 7) and were unlikely to be considered the cause of an epidemic.

DISCUSSION

The spread of ESBL-producing bacteria has been strikingly rapid worldwide, indicating that continuous monitoring systems and effective infection control measures are absolutely required. Therapeutic options for infections due to ESBL producers have also become increasingly limited. Health care interactions including the use of antibiotics, particularly oxyiminocephalosporins, and hospital transfer are among well-defined risk factors for acquisition of ESBL-producing bacteria (1, 2, 24). However, epidemiologic data and characterization of ESBL-producing isolates causing HA infection in Thailand are still rarely documented. Our results showed that a majority of patients were elderly and those who had urinary tract infections. It was not surprised that females had a higher prevalence of infection due to ESBL producers than males, since females are more vulnerable to urinary tract infection. ESBL-producing *K. pneumoniae* appeared to be an important cause of infection among patients in intensive-care units. All isolates remained susceptible to carbapenems. Besides high MICs of oxyiminocephalosporins, a majority of isolates were also resistant to non-β-lactam agents, especially tetracycline, gentamicin, cotrimoxazole, and ciprofloxacin, resulting in a very high percentage of MDR isolates. ESBL-producing *K. pneumoniae* generally appeared to be more resistant and showed an MDR phenotype at a higher rate than ESBL-producing *E. coli*.

Among 362 ESBL-producing isolates, 87.3% carried several *bla* genes, which can probably account for a high-level β-lactam-resistant phenotype. Although CTX-M types of ESBLs have been known for their rapid spread in Europe and Asia (6, 25), it was remarkable that in this study, 99.6% of ESBL-producing *E. coli* and 99.2% of ESBL-producing *K. pneumoniae* isolates carried $bla_{\text{CTX-M}}$. We therefore report the highest prevalence, to our knowledge, of $bla_{\text{CTX-M}}$ among ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* and demonstrate that CTX-M-type ESBL is highly endemic in Thailand. Interestingly, a survey at a university hospital in northeastern Thailand from 1994 to 1996 revealed that, among 32 isolates of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae*, bla_{SHV} was most predominant while $bla_{\text{CTX-M}}$ was not detected (11). The first detection of $bla_{\text{CTX-M}}$ in Thailand was later documented from a study of 48 *Enterobacteriaceae* isolates recovered during 1998 and 1999 at this hospital, where the prevalence of $bla_{\text{CTX-M}}$ was 52% (CTX-M-9 only) and subsequently increased to 65% among 52 isolates collected in 2003 (CTX-M-15, 44%; CTX-M-14, 11%; and CTX-M-9, 10%) (12). A survey in a neighboring country, Vietnam, during 2000 and 2001 demonstrated a low prevalence of ESBL-producing *E. coli* (4.4%) and ESBL-producing *K. pneumoniae* (3.0%) (9). In that series, CTX-M ESBLs were present in 18.7% of ESBL-producing *E. coli* and 61.5% of ESBL-producing *K. pneumoniae*, and CTX-M-14 was the only predominant type in which CTX-M-15 was not detected. Therefore, these surveys indicated not only geographic variations, but also increasing trends in the prevalence of CTX-M ESBLs.

In our study, a majority of ESBL-producing *E. coli* isolates possessed CTX-M-14, -15, and -55, while only CTX-M-14 and

-15 were predominant among ESBL-producing *K. pneumoniae*. CTX-M-55, a member of the CTX-M-1 subgroup, is a novel ceftazidime-resistant CTX-M recently reported by our group for its emergence in Thailand (20). This is also the first study to report CTX-M-27 and -40 in ESBL-producing *E. coli* and CTX-M-3 and -27 in ESBL-producing *K. pneumoniae* from Thailand. The fact that isolates carrying a gene of the CTX-M-1 subgroup significantly reduced susceptibility to tetracycline and cotrimoxazole and that the presence of other *bla* genes did not further increase the resistance rate may suggest that these resistance genes are often associated or cotransferred with $bla_{\text{CTX-M}}$. On the other hand, resistance to tetracycline and cotrimoxazole increased at a significant rate among CTX-M-9 subgroup-producing isolates only when other *bla* genes were present. Thus, these resistance genes are probably related to a gene in the CTX-M-9 subgroup at a greater distance. Further studies are needed to characterize plasmids that *bla* and other resistance genes are located on to gain a better understanding of their genetic relationships. IS*Ecp1*, a member of the IS*1380* family, is often located in the upstream region preceding $bla_{\text{CTX-M}}$, particularly in the CTX-M-1, -2, and -9 subgroups, and it probably plays a role in gene transfer or in providing a promoter for $bla_{\text{CTX-M}}$ (18, 23, 33). IS*Ecp1* was detected in most $bla_{\text{CTX-M}}$-carrying isolates in this study, supporting the rationale previously proposed that this IS element mainly mediated the dissemination of the gene. In addition, other IS elements, including IS*10*, IS*26*, and IS*903*, which was previously noted elsewhere to perhaps be involved in the mobilization of the $bla_{\text{CTX-M}}$ gene (6, 23, 34), were also found in some isolates. No IS element was detected in four $bla_{\text{CTX-M}}$-carrying isolates after several repetitions of the PCR experiment. There may be two reasons for this: (i) the IS element is located far upstream of $bla_{\text{CTX-M}}$, beyond the region covered by the primers used for PCR, or (ii) $bla_{\text{CTX-M}}$ mobilization may occur via an integron associated with a nearby gene on the transposable element, i.e., it did not require its own IS. The evidence that IS*Ecp1*s of different strains were found at various distances upstream from $bla_{\text{CTX-M}}$ is likely due to different insertion events and may support the first reason. It is noteworthy that four isolates of CTX-M-15-producing *E. coli* carried IS*26* within IS*Ecp1*, a characteristic reported to be a useful marker for isolates causing epidemics in the United Kingdom (39).

Other ESBL genes were less often encountered. Only 3.8% of ESBL-producing *E. coli* isolates carried bla_{SHV}, while 87.4% of ESBL-producing *K. pneumoniae* isolates carried bla_{SHV}. SHV-12 was the only SHV-type ESBL found among ESBL-producing *E. coli*. Among bla_{SHV}-carrying ESBL-producing *K. pneumoniae*, 51.4% of the genes encoded non-ESBL SHV-1 or -11 while the rest produced SHV-type ESBLs, including SHV-2a, -12, -27, -71, and -75. It is noteworthy that this study reported the detection of SHV-27-, -71-, and -75-producing isolates for the first time in Thailand. $bla_{\text{VEB-1}}$ was found associated with $bla_{\text{OXA-10}}$ in 32 out of 33 isolates, supporting the previous rationale that both genes are colocalized on the same class 1 integron (14, 26). The plasmid- or integron-mediated transfer was believed to cause the spread of $bla_{\text{VEB-1}}$ among enterobacterial isolates in Thailand (14), but its prevalence has rarely been studied. We report that $bla_{\text{VEB-1}}$ was detected in an average of 9.1% of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* isolates, which was less than a Vietnam report of 17.8% (9). OXA-10 is not an ESBL, while its derivatives, including OXA-11, -14, and -16, confer the ESBL phenotype by amino acid substitutions (7). Therefore, isolates in this series did not produce OXA-type ESBLs. The two *bla* genes encoding GES and PER β-lactamases were not detected in this study. The PFGE typing of ESBL-producing isolates showed various DNA banding profiles. Only a small number of isolates showed clonal relationships (>80% similarity) or were more distantly related (>60 to 80% similarity). A retrospective review of medical records suggested that these isolates did not significantly share patient demographics and occurrence periods. Generally, there was no significant difference in patient demographics and isolate characteristics between two hospitals studied. This clearly indicated that most ESBL-producing isolates were sporadic and that multiple clones were widespread in Thailand.

In summary, we report the first extensive study regarding the prevalence and molecular characterization of ESBL genes and the epidemiology of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* isolates causing HA infection in Thailand. This study clearly indicated that CTX-M, mainly CTX-M-14 and -15, and CTX-M-55 for ESBL-producing *E. coli* only, was highly endemic in Thailand. Most ESBL producers were resistant to oxyiminocephalosporins and other non-β-lactam agents at high levels and exhibited a high rate of the MDR phenotype. The spread of ESBL-producing bacteria appeared to be polyclonal, and none of the major epidemic strains were identified.

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